

## CLAIMS

1. A method for detecting, separating and identifying an expressed trace protein and/or peptide in a test sample, wherein a protein and/or peptide in a test sample is converted to a fluorescent derivative, said fluorescent derivative is separated by fluorescence detection, the fluorescent fraction is applied to mass spectrometry or the fluorescent fraction is applied to enzymatic hydrolysis, the peptide fragments are separated, and the fractions are applied to mass spectrometry, collated with a database and provided for structural analysis to identify the expressed protein and/or peptide.

2. The method according to claim 1, wherein after converting the protein and/or peptide in the test sample to a fluorescent derivative, the fluorescent derivative is applied to HPLC to capture the fluorescent fraction, the fluorescent fraction is applied to enzymatic hydrolysis, and fluorescence-labeled fragments and non-fluorescence-labeled fragments are applied to mass spectrometry or MS/MS analysis, and the ion molecular weight data of each of the fragments thus obtained is collated with a protein and/or peptide fragment database for structural analysis.

3. The method according to claim 1, wherein (1) the protein and/or peptide in the test sample is labeled with a fluorescence reagent, (2) the fluorescent fraction is captured by subjecting the labeled protein and/or

peptide to one-dimensional or two-dimensional HPLC/fluorescence detection, (3) the fluorescent fraction is applied to enzymatic hydrolysis, and (4) together with obtaining a fluorescence chromatogram by second stage HPLC/fluorescence detection of the resulting hydrolysis product, all of the peaks are applied to mass spectrometry and collated with a database for structural analysis.

4. The method according to any of claims 1 to 3, wherein a functional group-specific fluorescence reagent is added to an aqueous solution of the protein and/or peptide sample, and a surfactant and/or protein denaturing agent is optionally added, to fluorescently label the protein and/or peptide.

5. The method according to any of claims 1 to 3, wherein the fluorescence-labeled protein and/or peptide sample is applied to separation means typified by ion exchange column HPLC equipped with a fluorescence detector, reverse phase partition HPLC, gel filtration HPLC or electrophoresis, and the peak fraction thereof is captured while monitoring fluorescence.

6. The method according to any of claims 1 to 3, wherein the fluorescent fraction is subjected to enzymatic hydrolysis using a protease typified by various types of peptidases, trypsins and chymotrypsins.

7. The method according to any of claims 1 to 3,

wherein the enzymatic hydrolysis product is applied to reverse phase HPLC equipped with a fluorescence detector to detect a fluorescence peak, and mass spectrometry or MS/MS analysis is carried out on fluorescence-labeled fragments and non-fluorescence-labeled fragments.

8. The method according to any of claims 1 to 3, wherein ion molecular weight data of each fragment obtained by applying to mass spectrometry or MS/MS analysis is collated with a protein and/or peptide fragment database by a computer to analyze the structure and identify the protein and/or peptide prior to enzymatic hydrolysis.

9. The method according to any of claims 1 to 3, wherein the test sample is a protein and/or peptide sample collected from a biological sample.

10. The method according to any of claims 1 to 3, wherein database collation is carried out using a database containing protein and/or peptide fragment data and fluorescent reagent-labeled amino acid data.

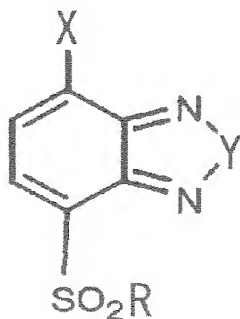
11. A system for detecting, separating and identifying an expressed trace protein and/or peptide used in the method according to any of claims 1 to 10 comprising, as constituent elements thereof, a first reactor for labeling a protein and/or peptide of a test sample with a fluorescence reagent, a one-dimensional or

two-dimensional HPLC equipped with a fluorescence detector for fluorescent fractionation of a fluorescent derivative labeled with the fluorescence reagent, a second reactor for enzymatic hydrolysis of the fluorescent fraction, a second-stage HPLC equipped with a fluorescence detector for fluorescent detection of fluorescence-labeled fragments of the enzymatic hydrolysis product, and one or two or more types of structural analysis devices equipped with a database containing data on amino acids labeled with the fluorescence reagent.

12. The system according to claim 11, wherein the first reactor, the one-dimensional or two-dimensional HPLC equipped with a fluorescence detector, the second reactor, and the second-stage HPLC equipped with a fluorescence detector are arranged in series.

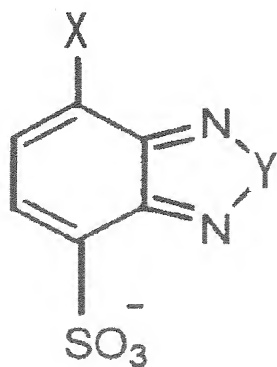
13. The method according to claim 1, wherein a protein and/or peptide in a test sample is converted to a fluorescent derivative by using as a fluorescent derivatization reagent a compound represented by the following general formula (1):

[Formula 1]



(wherein, X represents a halogen atom, Y represents O, Se or S, and R represents -NH<sub>2</sub>, -NHR' (wherein, R' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or -NR''R''' (wherein, R'' represents an alkyl group, and R''' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group)) or an isotope compound thereof, or a compound represented by the following general formula (2):

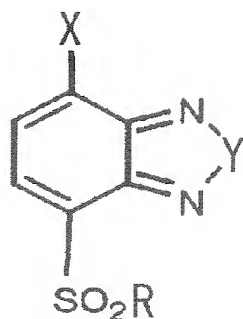
[Formula 2]



(wherein, X represents a halogen atom and Y represents Se or S), or an isotope compound thereof.

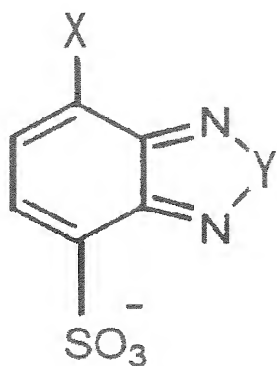
14. The method according to claim 1, wherein a protein and/or peptide in the method according to claim 1 is converted to a fluorescent derivative by using a fluorescent derivatization reagent used for fluorescent derivatization which is a compound represented by the following general formula (1):

[Formula 3]



(wherein, X represents a halogen atom, Y represents O, Se or S, and R represents  $-NH_2$ ,  $-NHR'$  (wherein,  $R'$  represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or  $-NR''R'''$  (wherein,  $R''$  represents an alkyl group, and  $R'''$  represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group)) or an isotope compound thereof, or a compound represented by the following general formula (2):

[Formula 4]



(wherein, X represents a halogen atom and Y represents Se or S), or an isotope compound thereof.

15. A method for detecting, separating and identifying a protein and/or peptide, wherein a protein and/or peptide of a test sample is converted to a

fluorescent derivative, the fluorescent derivative is separated and detected with an HPLC, enzymatic hydroxylation is carried out following fractionation, and sequence analysis and protein identification are carried out by direct mass spectrometry of the hydrolysis product.

16. A method for detecting, separating and identifying a protein and/or peptide, wherein a protein and/or peptide in different test samples in the form of sample A and sample B is converted to a fluorescent derivative, respectively, with at least two fluorescent derivatization reagents having different fluorescence wavelengths, the fluorescent derivative is separated and detected with an HPLC equipped with a fluorescence detector, and identification is carried out by applying to quantification of each fluorescence peak either directly or collectively following fractionation and/or applying each fluorescence peak collectively to enzymatic hydrolysis followed by quantification of the hydrolysis product, or applying the hydrolysis product to HPLC-mass spectrometry.

17. The method according to claim 16, wherein each fluorescence peak is applied to quantification by HPLC either directly or collectively, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

18. The method according to claim 16, wherein the

hydrolysis product is applied to quantification by HPLC, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

19. The method according to claim 16, wherein the reaction product of a first fluorescent derivatization reagent and the reaction product of a second fluorescent derivatization reagent with the protein and/or peptide in sample A and sample B are combined, applied to two HPLC capable of excitation and fluorescence detection, applied to enzymatic hydrolysis after fractionating and combining each fluorescence peak, and identification is carried out by applying the hydrolysis product to HPLC-mass spectrometry.

20. The method according to claim 16, wherein samples A and B are two types of cell, tissue or body fluid samples.

21. The method according to claim 16, wherein the protein and/or peptide is converted to a derivative with at least two fluorescent derivatization reagents having different excitation and fluorescence wavelengths among DAABD-X, DAASeBD-X and DAAThBD-X (wherein X represents Cl or F).

22. The method according to claim 21, wherein DAABD-X, DAASeBD-X or DAAThBD-X (wherein X represents Cl or F) and each isotope thereof are combined for use as



fluorescent derivatization reagents having different fluorescence wavelengths.

23. The method according to claim 16, wherein simultaneously with obtaining a peptide map by directly applying an enzymatically hydrolyzed sample to mass spectrometry, the structure of a peptide portion containing cysteine is acquired by utilizing the skeleton and electric charge of the fluorescence reagent and extracting fluorescence-labeled peptide fragments with a mass spectrometry measurement unit, and the protein and/or peptide is identified on the basis thereof.

24. An automated fractionation device capable of fractionating a protein and/or peptide derivatized with a fluorescent derivatization reagent without degrading the protein and/or peptide, at least provided with a microcolumn HPLC, microfluorescence detector, microfraction collector and automated microinjector.

25. A high-performance, easily quantifying trace protein identification and analysis device, at least provided with a microcolumn HPLC, microfluorescence detector, microfraction collector, enzyme reaction device and automated microinjector, and optionally provided with a mass spectrometry (MS) system.